It is observed, as in the preceding trial, that the separation is more rapid at a higher temperature, but that the gain in resolution is lower than with for example T15 or T7, which exhibit a more marked thermothickening character. This confirms that the thermothickening character, as it appears in the viscosity curves as a function of the temperature, is a characteristic advantage of the media according to the invention, and that the said viscosity curves may be used as a guide for optimizing the separation properties.

Trial 4-5

Separation of the sequence products using a separation medium comprising the copolymer PAM-NIPAM (T10).

The copolymer is used at two different concentrations, 3 g/100 ml and 5 g/100 ml, respectively, in TRIS-TAPS buffer (50 mM) containing 7M urea. The pH of the medium is of the order of 8.2. As a control, a trial is carried out with a commercial sequencing medium (POP6 Perkin-Elmer), used as received.

The capillary used has a length of 40 cm, with an effective length of 30 cm and an internal diameter of 100 µm. For the separation media based on T10 polymer, which do not exhibit specific properties of adsorption on silica, the capillary is, prior to its use, washed with a 1M hydrochloric acid solution and comprising 1 g/100 ml of polyvinylpyrrolidone, having a molecular mass of 1 000 000. The separation medium is introduced into the capillary at 25°C.

The sample tested is the product of a reaction of sequence of DNA ssM13mp18, (fragments with "T" ending), prepared by cyclic sequencing with the fluorescein-primer kit distributed by Amersham, according to the instructions provided by the manufacturer.

The electric field is 200 volts per centimetre and the injection is carried out over 8 seconds at 200 volts per centimetre. The separating capacities of each of these media are evaluated at the temperature of 60°C. Figures 6 and 7 present the separating capacities of the commercial sequencing medium (control Figure a), and the media according to the invention based on the copolymer T10 at the respective concentrations of 3 g/100 ml (Figures b) and 5 g/100 ml (Figures c) (the numbers above the peaks represent the length of the DNA fragment minus 48 bases).

Trial 4-6

Separating properties with respect to fragments of sequences using various separation media in accordance with the invention.

The media tested are based either on the copolymer PAM-NIPAM (T12) at a concentration of 8 g/100 ml, on the copolymer PAM-NIPAM (T13) with a concentration of 5 g/100 ml or on the copolymer DMAM-NIPAM (T7) with a concentration at 5 g/100 ml, respectively.

Each of these media is of course supplemented with TRIS-TAPS buffer (50 mM) and with 7M urea. They have a pH of 8.2, the nature of the sample and the separation conditions are the same as those chosen in the context of the preceding trial.

These different trials confirm that several media according to the invention, having a relatively moderate viscosity at room temperature of the order of 1000 $mPa \cdot m^{-1} \cdot s^{-1}$, or even markedly lower, make it possible to separate the DNA sequence fragments with performances equal to or greater than those of the commercial media. It will be observed in particular that the resolution obtained with T10 at 5 g/100 ml is markedly greater than that obtained with POP6, in a slightly shorter time.

Trial 4-7

Separation using DNA fragments of a medium comprising, as copolymer, the copolymer PAM-NIPAM (T10).

The copolymer used at a concentration of 2 g/100 ml is mixed with the TRIS-TAPS buffer (50 mM), 2 mM EDTA and with the DNA marker SYBR GREEN I 10^{-4}. The length of the capillary is 15 cm, of which 10 cm up to the detector, and the capillary is of the "DB17" type (JW scientific) having an internal diameter of 100 micrometres, so as to eliminate the residual electroosmosis which appears with this medium.

In this particular case, the sample is the marker High molecular weight standard (Life Technologies), and has fragments between 8 271 and 48 502 base pairs. The injection is carried out over 5 seconds at 100 volts per centimetre. The separation is carried out in a pulsed field with square pulses of +/- 200 V, an asymmetry between the + and - pulses of 20% and a frequency of 30 Hz. The separation of the fragments may be carried out in less than 20 min (Figure 8), against several hours in an ordinary entangled polymer solution (Heller et al. Electrophoresis, 16, 1423-1428 (1995)).

Trial 4-8

Separation and resolution properties at 40°C of separation media in accordance with the invention.

The media tested contain polymers based on NIPAM macromonomers of different length (T22, T26, T24 and T25, respectively), dissolved at 3 g/100 ml in TRIS-TAPS buffer (50 mM) containing 7M urea. The pH of the medium is of the order of 8.2. The sample used is "50 BL LADDER-fluorescein", Pharmacia.

The results, in terms of peak width, are given in Figure 12.